CLAIMS

1. Use in a diagnostic hybridisation assay of a probe for lowering the effect of sequence variations in a nucleic acid analyte, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, **characterized in that** the probe comprises one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification and the diagnostic assay is for assessing the amount of analyte present in the sample.

2. Use in a diagnostic hybridisation assay of a probe for lowering the effect of sequence variations in a nucleic acid analyte, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, **characterized in that** the probe comprises one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, i.e. at a constant temperature of hybridisation, the melting temperature of the probe with any possible analyte's polymorphism is increased compared to the melting temperature of an unmodified probe with any analyte's polymorphism and the diagnostic assay is for assessing the presence of the analyte in the sample.

3. Use as claimed in claims 1-2, wherein the probe is a molecular beacon.

4. Use in a diagnostic hybridisation assay of a

molecular beacon probe for lowering the IBL effect due to the possible opening of the stem of the molecular beacons by way of at least one contaminant present in the amplification enzymes mixture, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, **characterized in that** the probe's stem comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, especially 2'-*O*-methyl nucleotides, and
- one or more unmodified nucleotides.

5. Use in a diagnostic hybridisation assay of a probe for lowering:

- the effect of sequence variations in a nucleic acid analyte, and/or
- the IBL effect due to the possible opening of the stem-loop structure of the molecular beacons by way of at least one contaminant present in the amplification enzymes mixture,

which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, **characterized in that** the probe's loop comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and
- one or more unmodified nucleotides.

and/or the probe's stem comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, especially 2'-*O*-

methyl nucleotides, and

- one or more unmodified nucleotides.

6. Use as claimed in any one of the claims 1-5 wherein the diagnostic assay is a homogeneous assay.

7. Use as claimed in any one of the claim 1-5 wherein the diagnostic assay is a heterogeneous assay.

8. Use as claimed in any one of the claims 1-7, wherein the nucleotides or nucleotide analogues having an affinity increasing modification are selected from the group consisting of 2'-O-derivatized nucleotides, locked nucleic acids and peptide nucleic acids.

9. Use as claimed in claim 8, wherein the 2'-O-derivatized nucleotide is a 2'-O-methyl-nucleotide.

10. Probe for use in a diagnostic hybridisation assay, said probe comprises one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification i.e. at a constant temperature of hybridisation, the melting temperature of the probe with any possible analyte's polymorphism is increased compared to the melting temperature of an unmodified probe with the same target.

11. Molecular beacon probe for use in a diagnostic hybridisation assay, said probe allowing the lowering of the IBL effect, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, characterized

in that the probe's stem comprises:

- one or more 2'-*O*-methyl nucleotides, and
- one or more unmodified nucleotides.

12. Molecular beacon probe for use in a diagnostic hybridisation assay, said probe allowing the lowering of:

- the effect of sequence variations in a nucleic acid analyte, and/or
- the IBL effect due to the possible opening of the stem-loop structure of the molecular beacons by way of enzymes,

characterized in that the probe's loop comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and
- one or more unmodified nucleotides.

and/or the probe's stem comprises:

- one or more 2'-*O*-methyl nucleotides, and
- one or more unmodified nucleotides.

13. Probe or molecular beacon probe as claimed in any one of the claims 10-12, wherein the nucleotides or nucleotide analogues having an affinity increasing modification are selected from the group consisting of 2'-O-derivatized nucleotides, locked nucleic acids, peptide nucleic acids.

14. Probe or molecular beacon probe as claimed in claim 13, wherein the 2'-O-derivatized nucleotide is a 2'-*O*-methyl-nucleotide.

15. Molecular beacon probe as claimed in any one of the claims 11-14, wherein each base pair constituting the stem contains no more than one 2'-*O*-methyl nucleotide.

16. Molecular beacon probe as claimed in any one of the claims 11-15, wherein at least one base pair constituting the stem contains no nucleotide or nucleotide analogue having an affinity increasing modification.

17. Molecular beacon probe as claimed in any one of the claims 11-16, wherein one base pair constituting the stem contains no nucleotide or nucleotide analogue having an affinity increasing modification

18. Molecular beacon probe as claimed in any one of the claims 11-17, wherein each strand constituting the stem contains at least one nucleotide or nucleotide analogue having an affinity increasing modification.

19. Kit for performing a diagnostic amplification assay, comprising the appropriate primers, polymerase(s) and reagents for performing amplification of an analyte to be diagnosed and a probe or a molecular probe as claimed in claims 10-18 for detecting the amplified analyte.